The effect of subunit or modified live bovine herpesvirus-1 vaccines on the efficacy of a recombinant *Pasteurella haemolytica* vaccine for the prevention of respiratory disease in feedlot calves

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Abstract

The efficacy of a *Pasteurella haemolytica* vaccine (PhV) administered once to calves within 24 hours of arrival at a feedlot was tested for the ability to prevent morbidity and mortality from all bovine respiratory disease (BRD) and specifically from fibrinous pneumonia mortality. The PhV consisted of two immunizing ingredients: outer membrane proteins extracted from *P. haemolytica*, plus genetically attenuated leukotoxin produced by recombinant DNA technology. This double blind study was conducted at a large Saskatchewan feedlot using 2,324 high-risk calves purchased at auction markets and kept under typical commercial feedlot conditions. The trial design included four vaccine test groups: 1) PhV and a bovine herpesvirus type-1 (BHV-1) subunit vaccine comprised only of the virus glycoprotein IV (gIV); 2) PhV and a commercial modified live vaccine (MLV) containing BHV-1 and parainfluenza-3 viruses; 3) gIV alone; and 4) MLV alone. Calves were assigned to vaccine groups in a random systematic manner, individually identified, and monitored for 90 days after vaccination. The vaccines were given once, on arrival, to reflect common feedlot practice, although vaccination prior to expected risk would be more appropriate.

The PhV in combination with gIV reduced BRD morbidity by 20% ($p < 0.05$) compared to gIV alone and 24% ($p < 0.05$) compared to MLV alone, and reduced BRD mortality by 88% ($p < 0.05$) and fibrinous pneumonia mortality by 100% ($p < 0.05$) when compared to either gIV or MLV alone. Vaccination with PhV in combination with MLV significantly reduced the efficacy of the PhV in preventing BRD morbidity, BRD mortality, and fibrinous pneumonia mortality and also reduced the antibody response to *P. haemolytica* leukotoxin. These results suggest that the MLV interfered with the protective capacity of the PhV.

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Résumé

Les effets du vaccin vivant modifié ou de sous-type antiherpès-1 d'origine bovine sur l'efficacité du vaccin par recombinaison contre *Pasteurella haemolytica* pour la prévention de maladies respiratoires chez les veaux en parc d'engraissement

Cette étude porte sur l'efficacité du vaccin anti *Pasteurella haemolytica* (VPh) administré en une dose unique chez les veaux dans les 24 heures suivant leur arrivée au parc d'engraissement. Le vaccin a été évalué quant à sa capacité à prévenir la morbidité et la mortalité reliées à toutes les infections respiratoires bovines (IRB) et plus particulièrement, la mortalité due à la pneumonie fibrineuse. Le vaccin renferme deux substances immunisantes, soit des protéines extraites de la membrane externe de *P. haemolytica* et une leucotoxine modifiée par recombinaison génétique de l'ADN. Cette étude à double insu a été effectuée dans un parc d'engraissement de la Saskatchewan et compte 2,324 veaux à risque élevé, achetés à l'encan et gardés dans un environnement typique des parcs d'engraissement commerciaux. Les animaux ont été répartis au hasard en quatre groupes selon un protocole d'immunisation : groupe 1, le vaccin anti *Pasteurella haemolytica* (VPh) et le vaccin antiherpès-1 d'origine bovine (VHB-1) de sous-type fait seulement à partir de la glycoprotéine IV du virus (gIV); groupe 2; le vaccin VPh et le vaccin commercial vivant modifié combiné VHB-1/parainfluenza-3 (VVM); groupe 3, le vaccin gIV seulement; groupe 4, le vaccin commercial vivant modifié combiné VHB-1/parainfluenza-3. Chaque animal a été identifié et observé sur une période de 90 jours postimmunisation. Les vaccins ont été administrés en une seule dose dès l'arrivée, afin de reproduire le protocole usuel utilisé dans les parcs d'engraissement commerciaux en dépit du fait que l'administration de la vaccination serait plus appropriée dans la période précédant les facteurs de risque.

Les résultats indiquent que l'immunisation combinée des vaccins VPh et gIV a réduit la morbidité des infections respiratoires bovines de 20 % ($p < 0,05$) comparativement à l'administration unique du gIV et de 24 % ($p < 0,05$) comparativement à l'administration unique du VVM. De plus, ce protocole a réduit la mortalité des IRB de 88 % ($p < 0,05$) et celle reliée à la pneumonie fibrineuse de 100 % ($p < 0,05$) comparativement aux protocoles utilisant soit le gIV ou le VVM seul. L'immunisation combinée VPh-VVM a réduit de façon significative l'efficacité du VPh dans la prévention de la morbidité des IRB; de la mortalité

des IRB et de la pneumonie fibrineuse et a aussi réduit la stimulation immunologique contre la leucotoxine de *P. haemolytica*. Ces résultats suggèrent que le VVM interfère avec la capacité protectrice du VPh.

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Introduction

Losses due to bovine respiratory disease (BRD) are some of the largest health costs incurred during the fattening of feedlot calves (1-7), with fibrinous pneumonia attributed to *Pasteurella haemolytica* being the most important cause of BRD mortality (5-7). Vaccines for preventing BRD have been investigated for more than 70 years (8). Vaccination on arrival to prevent both bacterial and viral BRD pathogens is widely used in feedlots. However, several reports indicate that vaccines to prevent BRD are either ineffective or produce conflicting results under field conditions (8-14). Therefore, improved vaccines for *P. haemolytica* and other pathogens involved in the BRD complex are still required.

During the last decade, considerable progress has been made toward defining the virulence factors that allow *P. haemolytica* to cause disease. This has also led to the identification of protective components that can be used to develop effective vaccines. A leukotoxin secreted by *P. haemolytica* is an important element in the pathogenesis of fibrinous pneumonia (15,16). It has been shown that the production of antibodies to leukotoxin can prevent or reduce the occurrence of fibrinous pneumonia in experimentally challenged calves (17,18). We have shown that a recombinant DNA form of leukotoxin produced by a genetically engineered strain of *Escherichia coli* also protects calves from experimentally induced *P. haemolytica* pneumonia (19,20). This genetically attenuated leukotoxin has no toxic activity but retains the portion of the molecule required to stimulate the production of leukotoxin-neutralizing antibodies. More recently, leukotoxin produced by recombinant methods appeared to increase protection against experimental challenge with *P. haemolytica* provided by a vaccine prepared from the supernatant of log phase culture (21). Vaccination with extracts of outer membrane proteins of *P. haemolytica* have also been shown to be protective against experimental challenge (19,20,22).

Acute fibrinous pneumonia caused by *P. haemolytica* is often the final stage of BRD. Factors, including stressors and several viruses, may increase the susceptibility of the lung to this bacterial infection. Bovine herpesvirus-1 (BHV-1) and parainfluenza-3 virus (PI-3) are thought to be two of the important viral initiators of BRD (23). However, vaccinating feedlot calves with BHV-1 and PI-3 virus vaccines does not reduce the overall incidence of BRD (9), although there is evidence that they can prevent epidemics of infectious bovine rhinotracheitis (IBR) (24, unpublished observations). Therefore, vaccines containing BHV-1 and PI-3 are administered to feedlot calves, and are often given concurrently with vaccines for the prevention of pasteurellosis and other bacterial diseases. Both modified live and killed BHV-1 vaccines are available but most feedlots use the modified live virus vaccines. Modified live virus BHV-1 vaccines may reduce the incidence of IBR, but there is also evidence that they may increase the risk of BRD morbidity (11,27) and mortality (11,25,26) in cattle. However, the mechanisms by which this occurs have not been identified. Attenuated vaccines against other viruses have been shown to suppress the number and activity of peripheral blood monocytes (28) and to depress T cell responsiveness (29), but this possibility has not been explored with BHV-1 vaccines. In addition, some vaccine strains of BHV-1 may have caused IBR outbreaks under certain circumstances (27). To overcome some of these risks, killed BHV-1 virus vaccines have been advocated, but their efficacy in preventing IBR has been questioned (30,31). Therefore, the types of BHV-1 vaccines which are currently available do not appear to be ideal for use in feedlot cattle.

As an alternative to the use of modified live and killed BHV-1 vaccines, we have explored the use of individual BHV-1 glycoproteins as subunit vaccines. Bovine herpesvirus-1 is comprised of approximately 30 different proteins. However, only a few of them stimulate the formation of protective immunity. Subunit vaccines which contain BHV-1 glycoproteins I, III or IV, used alone or in combination, have been shown to protect calves against experimental challenge with BHV-1 followed by *P. haemolytica* (31,32). Subunit vaccines comprised of specific viral proteins may provide an effective alternative to the currently used modified live or killed virus vaccines.

In this study, we report the efficacy of a *P. haemolytica* vaccine (PhV) that contains an extract of outer membrane proteins plus leukotoxin produced by recombinant DNA technology, administered once to calves upon arrival at a commercial feedlot. Since most feeder calves are also vaccinated against IBR upon arrival, we also wanted to examine the impact of two types of viral vaccines when given at the same time as the PhV. One viral vaccine was an experimental subunit vaccine that contained only glycoprotein IV of BHV-1 (gIV), and the other one a commercial modified live virus vaccine (MLV) which contained BHV-1 and PI-3. These vaccines were tested using a 2 × 2 factorial design. The objective was to examine the efficacy of PhV when administered alone or concurrently with either gIV or MLV within 24 h of arrival in reducing subsequent BRD morbidity, mortality, and fibrinous pneumonia mortality in high-risk calves in a commercial feedlot. Calves in these groups were compared to control groups which received either gIV alone or MLV alone.

Materials and methods

Vaccines

For the preparation of PhV, genetically attenuated leukotoxin was prepared from *E. coli* as inclusion bodies which were solubilized in 4 M guanidine hydrochloride and refolded by gentle dialysis against 100 mM Tris-buffered saline, pH 7.5. The purity of

the leukotoxin was determined by denaturing polyacrylamide gel electrophoresis (33). *Pasteurella haemolytica* surface antigens were extracted by vigorously shaking log-phase cells in NaCl for 1 h (34,35). Less than 1% of the cells broke and leaked intracellular components as determined by beta-galalactosidase activity. The cells were removed by centrifugation at 6,000 × *g* for 10 min and the supernatant was passed through a 0.45 micron cellulose acetate membrane. Protein concentration was measured with a colorimetric test (Biorad protein assay, Biorad Laboratories, Mississaga, Ontario). The antigens were combined with an oil-based adjuvant so that each dose of the final PhV contained 100 μg of genetically attenuated leukotoxin and 50 μg of protein extract in a total volume of 2 mL.

It was a standard practice in the feedlot where this study was conducted to vaccinate all calves against BHV-1 at arrival, because of previous outbreaks of IBR. The managers of this feedlot did not want to leave any calves unvaccinated against BHV-1. Therefore, the control population in this trial consisted of calves vaccinated according to the standard practice for this feedlot using a vaccine containing modified live BHV-1 and PI-3 virus (Bovilan RP, Langford Inc., Guelph, Ontario). For comparison, a BHV-1 subunit vaccine containing only glycoprotein IV (gIV) produced in an insect cell system was used (36). This vaccine contained the same oil-based adjuvant as was used in the PhV and was administered in a 2 mL dose.

Trial design

This study was conducted at a large, 10,000 head capacity commercial feedlot in central Saskatchewan during the fall and winter of 1989–1990. Recently-weaned calves, approximately 7–10 months of age and weighing between 250 and 350 kg, were purchased from auction markets in Alberta, Saskatchewan, and Manitoba, and transported to the feedlot in trucks holding approximately 75 calves each. Each truckload came from a single auction market and included calves from different farms or ranches. Within 24 h of arrival at the feedlot, each group of calves was processed, which involved ear tagging with a unique number, branding, injection with 5 mL of vitamins A and D (Poten AD, rogar/STB, Pointe Claire-Dorval, Quebec) and with 3 mL of ivermectin (Ivomec, MSD Agvet, Kirkland, Quebec), as well as implanting with zeranol (Ralgro, I.M.C., Terre Haute, Indiana, USA). All calves were vaccinated with 5 mL of a combined *Haemophilus somnus* and clostridial bacterin (Fermicon 7/Somnugen, Boehringer Ingelheim Animal Health, Burlington, Ontario). Rectal temperatures were taken in all calves at processing and any animal with a temperature ≥ 40.5°C was treated for BRD with antibiotics and excluded from the trial. All other calves were treated prophylactically with 5,000–6,000 mg of long-acting oxytetracycline (Liquamycin/LA, rogar/STB).

		gIV (sub-unit BHV-1) vaccine	MLV (BHV-1, PI-3) vaccine
Pasteurella haemolytica vaccine	Total n=	585	583
	BRD morbidity	119	140
	BRD mortality	1	5
	Fib.pneumonia mortality	0	5
No *Pasteurella haemolytica* vaccine	Total n=	579	577
	BRD morbidity	147	154
	BRD mortality	8	8
	Fib.pneumonia mortality	6	6

Figure 1. A 2 × 2 table with the number of calves assigned to each group and the number of calves in each group with BRD morbidity, BRD mortality, and fibrinous pneumonia mortality.

Calves from each arriving truckload group were assigned to experimental vaccine groups in a random systematic manner as they proceeded through the chute at processing. The experimental groups were: 1) PhV and gIV; 2) PhV and MLV; 3) gIV only; and 4) MLV only (Figure 1). All vaccines were administered intramuscularly. When two vaccines were given concurrently, separate but adjacent injection sites were used. After processing, the entire arriving group of calves was placed in a pen. Further groups were added until the pen contained between 200–300 calves. Thus, each pen had approximately the same number of calves from each of the four experimental treatment groups. In total, 2,324 calves were included in this trial (Figure 1).

Calves were observed daily for illness by experienced pen riders checking for sick animals. Calves were selected as BRD cases when they appeared depressed

Table 1. Risks[a] of morbidity and mortality

Vaccine	BRD[b] morbidity	Fibrinous pneumonia mortality	BRD mortality	*H. somnus* mortality	All mortality
PhV[c] and gIV[d]	20.3	0	0.2	1.0	2.1
gIV	25.4	1.0	1.4	1.2	2.8
PhV and MLV[e]	24.0	0.9	0.9	1.5	2.7
MLV	26.7	1.0	1.4	1.4	3.3
Overall	24.1	0.7	0.9	1.3	2.7

[a]Risk = (Number of cases in group/number of calves in group at start) × 100%
[b]Bovine respiratory disease
[c]*P. haemolytica* vaccine
[d]Subunit BHV-1 vaccine
[e]Modified live BHV-1 vaccine

Table 2. Relative risks (RR)[a] for fibrinous pneumonia mortality[b], BRD mortality[c], and BRD morbidity[d]

Test vaccine group[e]	Control vaccine group[f]	Disease	RR	95% CI[g]	p
PhV[h] and gIV[i]	MLV[j]	Fib. pna.[b]	0	n/a	0.01
		BRD mort.[c]	0.12	0.02,0.98	0.02
		BRD morb.[d]	0.76	0.62,0.94	0.01
gIV	MLV	Fib. pna.	1.00	0.32,3.07	1.00
		BRD mort.	1.00	0.38,2.64	1.00
		BRD morb.	0.95	0.78,1.16	0.61
PhV and MLV	MLV	Fib. pna.	0.82	0.25,2.70	0.75
		BRD mort.	0.62	0.20,1.88	0.39
		BRD morb.	0.90	0.74,1.10	0.29
PhV and gIV	gIV	Fib. pna.	0	n/a	0.01
		BRD mort.	0.12	0.02,0.99	0.02
		BRD morb.	0.80	0.65,0.99	0.04
PhV and gIV	PhV and MLV	Fib. pna.	0	n/a	0.03
		BRD mort.	0.20	0.02,1.70	0.12
		BRD morb.	0.85	0.68,1.05	0.13

[a]Relative risk = (risk for the test group/risk for the control group)
[b]All deaths in which fibrinous pneumonia was the primary diagnosis
[c]All deaths where respiratory disease was the primary diagnosis
[d]All cases that were treated because of a primary diagnosis of bovine respiratory disease
[e]The numerator in the relative risk
[f]The denominator in the relative risk
[g]95% precision-based confidence interval of the relative risk
[h]*P. haemolytica* vaccine
[i]Subunit BHV-1 vaccine
[j]Modified live BHV-1 vaccine

or inappetent, and/or if they had symptoms attributable to the respiratory system, and they had to have a rectal temperature ≥ 40.0°C. A complete postmortem examination was performed within 24 h of death by a veterinarian on calves that died. Samples for histological and microbiological examination were submitted to the diagnostic pathology laboratory at the Western College of Veterinary Medicine only when necessary to confirm the gross necropsy diagnosis. The feedlot employees selecting the sick calves and the veterinarians making the postmortem diagnoses were unaware of the vaccination status of the individual calves.

The cause of death was classified by primary gross lesions into several categories (histological and microbiological findings were used when gross diagnosis was not definitive). The category of "BRD mortality" included all calves that died from pneumonia of any type (fibrinous pneumonia, fibrinous-bronchopneumonia, bronchopneumonia, and interstitial pneumonia). "Fibrinous pneumonia mortality" included all the calves that died with typical lesions of fibrinous pneumonia or fibrinous-bronchopneumonia (37). "*Haemophilus somnus* mortality" included those calves that died of myocarditis, pleuritis, septicemia, thrombotic meningoencephalitis (TME), and/or polyarthritis (38–40), but not those cases of pneumonia where *H. somnus* may have been a contributing cause.

Blood samples were collected twice from approximately 5% of the calves: once at the time of initial vaccination, and from the same calves 30 days later. In each arriving group, one calf from each vaccine group was randomly selected for blood sampling. Whole blood was collected in 10 mL vacuum tubes (Vacutainer, Becton Dickinson, Mississauga, Ontario) and stored at 4°C for 24 h. The blood was then centrifuged at 5000 × *g* for 10 min and approximately 3 mL of serum were removed from each tube and stored frozen at −70°C until assayed.

Serology

Serum antileukotoxin enzyme-linked immunosorbent assay (ELISA) titers were determined on all samples collected. Purified recombinant leukotoxin was diluted in 50 mM carbonate buffer, pH 9.5, to a final concentration of 10 μg/mL and 100 μL added to each well of a 96-well plate (Microtiter Plate, Dynastar Laboratories Immunlon II, Baxter/Canlab, Toronto, Ontario). After 24 h at 4°C, plates were washed four times with distilled water and fourfold serial dilutions of the test sera were made in each row of the plate. Plates were incubated at room temperature for 1 h and washed eight times in distilled water. The second antibody (Goat antibovine IgG, alkaline phosphatase conjugate, 500 μg/mL, Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA) was diluted 7500-fold and 100 μL added to each well. After 1 h, plates were washed eight times and phosphatase activity measured colorimetrically with a commercial kit (ELISA Amplification System, Gibco/BRL, Mississauga,

Table 3. Vaccine efficacy (VE)[a] for the reduction of fibrinous pneumonia mortality[b], BRD[c] mortality, and BRD morbidity

Test vaccine group	Control vaccine group	Fibrinous pneumonia mortality VE	Fibrinous pneumonia mortality 95% CI[d]	BRD mortality VE	BRD mortality 95% CI	BRD morbidity VE	BRD morbidity 95% CI
PhV[e] and gIV[f]	MLV[g]	100%	20%,100%	88%	15%,100%	24%	6%,42%
PhV and gIV	gIV	100%	20%,100%	88%	15%,100%	20%	1%,39%
PhV and gIV	PhV and MLV	100%	13%,100%	n/a[h]	n/a	n/a	n/a

[a]The proportion of the disease in the control calves that was attributable to their not being vaccinated with the experimental vaccine (40)
[b]Includes all deaths in which fibrinous pneumonia was the primary diagnosis
[c]Bovine respiratory disease
[d]95% precision-based confidence interval of the vaccine efficacy
[e]*P. haemolytica* vaccine
[f]Subunit BHV-1 vaccine
[g]Modified live BHV-1 vaccine
[h]Not calculated because risk in test group not significantly different from risk in control group (Table 2)

Ontario). The background was calculated by taking the mean absorbance plus two standard deviations of the negative control wells. The titer of each sample was expressed as the reciprocal of the final dilution which resulted in a positive value relative to the background. Antileukotoxin ELISA titers determined in this manner have been shown to correlate directly with leukotoxin neutralizing titers (20). Serum antibody to BHV-1 glycoproteins gI and gIV were determined by a modified ELISA technique described previously (41).

Analysis

The unit of analysis for disease outcomes was the individual animal. All disease levels were expressed as risks (Table 1). The groups that received PhV were compared to those that did not receive PhV (Table 2) and gIV groups to those that received MLV. Relative risks (RR) (Table 2) and 95% precision-based confidence intervals (95% CI), and Mantel-Haenszel chi-square p values were calculated (42). The Breslow-Day test (Pro Freq, SAS User's Guide: Statistics, SAS Stat Guide, Gary, North Carolina, USA) was used to assess the homogeneity of the odds ratio across strata (BHV-1 vaccine status). Summary RR were not calculated because of a lack of homogeneity. Vaccine efficacy (VE) is the proportion of disease in control calves attributable to their being unvaccinated (43). When the RR was significantly different between groups (the 95% CI did not include unity), the VE and its precision-based 95% CI were calculated.

The serological data were analyzed by the Statistical Analysis System computer program (SAS Institute, Box 8000, Cary, North Carolina, USA). All titers were expressed as the $\log_2$ reciprocal of the end point and results were analyzed by analysis of variance. The model for the day 0 and day 30 geometric mean titers included the categorical independent variables for PhV and IBR vaccination. The titer at day 0 was also included as a continuous independent variable for the models describing the geometric mean titers for day 30. The Tukey-Kramer method was used for the comparison of means for main effects (PhV and MLV) (Proc GLM, SAS Stat Guide). Least-squares mean titers (LSM) were determined for each of the four vaccine groups. The probability values for the null hypothesis that the LSM for any group was equal to any other group's LSM [H_0: LSM(i) = LSM(j)] (Proc GLM, SAS Stat Guide) were determined.

Results

Calves in this trial had an overall risk of treatment for BRD of 24.1%, a risk of dying from BRD of 0.9%, and a risk of dying of fibrinous pneumonia of 0.7% (Table 1). Other causes of death included hemophilosis, bloat, peritonitis, nervous coccidiosis, endocarditis, anaphylactic shock, and ruptured eosophagus. The crude (or overall) mortality risk was 2.7% and the risk for mortality due to *H. somnus* was 1.3%. These disease incidences were similar to those reported previously for feedlots in western Canada (1,2), except for the increased incidence of hemophilosis, which has been noted in some recent papers (38–40).

When used in combination, PhV and gIV significantly reduced BRD morbidity by 20% ($p < 0.05$) when compared with vaccination with just gIV, and by 24% ($p < 0.05$) when compared to vaccination with just MLV (Table 3). The same vaccine combination reduced BRD mortality by 88% ($p < 0.05$), and fibrinous pneumonia mortality by 100% ($p < 0.05$) when compared to vaccination with either gIV alone or MLV alone (Table 3). However, this vaccine regimen did not significantly effect the *H. somnus* mortality, nor other causes of mortality or overall mortality. Vaccination with PhV in combination with MLV showed no significant improvement in disease outcome over vaccination with just MLV or with just gIV. Fibrinous pneumonia mortality was significantly lower in the PhV with gIV group than in the PhV with MLV group ($p<0.05$) (Tables 2 and 3). The Breslow-Day test for homogeneity between strata indicated significant differences in odds ratios between strata (MLV vs gIV) for fibrinous pneumonia mortality.

The PhV significantly increased, and the MLV significantly reduced antileukotoxin titers ($p < 0.05$). Calves that received PhV in combination with gIV had

Table 4. Least-squares geometric mean ELISA titers

Vaccine group	Day 0[a]	Day 30[b]
Leukotoxin		
PhV[c] and gIV[d]	3999[e]	38520[e]
gIV	3646[e]	17050[fg]
PhV and MLV[h]	3249[e]	22993[f]
MLV	3324[e]	13771[g]
BHV-1[i] gIV glycoprotein		
PhV and gIV	1.8[f]	68.0[f]
gIV	1.7[f]	47.7[f]
PhV and MLV	2.3[f]	74.8[f]
MLV	1.3[f]	71.4[f]
BHV-1 gI glycoprotein		
PhV and gIV	1.2[f]	3.1[f]
gIV	1.6[fg]	2.4[f]
PhV and MLV	2.6[f]	103.8[g]
MLV	1.3[fg]	40.8[g]

[a]Day of vaccination
[b]Titer 30 days after vaccination
[c]*P. haemolytica* vaccine
[d]Subunit bovine herpes virus-1 vaccine
[e,f,g]With different superscripts in a column, means are significantly different ($p < 0.05$), ANOVA with Tukey-Kramer means test
[h]Modified live bovine herpesvirus-1 vaccine
[i]Bovine herpesvirus-1

significantly higher antileukotoxin titers than any of the other groups (Table 4). The group vaccinated with PhV and MLV had significantly higher titers than the group that received MLV only, but they were not significantly higher than the group that received only gIV (Table 4). There were no significant differences between groups in antibody titers to the BHV-1 gIV glycoprotein on day 30 (Table 4).

There were no obvious clinical or postmortem indications of IBR disease in any of the calves on this trial. Only 12.5% of all the calves had positive ELISA titers to the BHV-1 gI glycoprotein on arrival at the feedlot (Table 4). A further 71.7% of the MLV-vaccinated calves became seropositive for BHV-1 gI by day 30, but only a further 18.3% of the gIV vaccinated calves became seropositive to BHV-1 gI (Table 4). None of the calves vaccinated with PhV or gIV showed any adverse reactions.

Discussion

In this study we have tested the safety, efficacy, and compatability of a new recombinant *P. haemolytica* vaccine with two types of BHV-1 vaccines, administered once at entry to the feedlot. Although the recommended vaccination protocol should include two vaccinations, at least three weeks apart and 10 days prior to exposure (or feedlot entry), we have demonstrated that a *P. haemolytica* vaccine given at the same time as a BHV-1 glycoprotein IV subunit vaccine reduced BRD morbidity, and mortality caused by BRD and fibrinous pneumonia. While this protocol is not ideal, vaccination is commonly done this way in most large western Canadian feedlots because of convenience and the early occurrence of BRD upon entry to the feedlot.

The multifactorial design used in this study allowed the analysis of several variables and their interactions, while also having the advantage of increased power over several independent trials for each variable (43). The analysis of the data in this trial provided several indications that there was an interaction between the BHV-1 vaccines and the PhV. There was a lack of homogeneity between BHV-1 vaccine stratas (Breslow-Day test) when analyzing mortality due to fibrinous pneumonia. The PhV vaccine was effective in preventing BRD mortality and morbidity when it was given with gIV, but not when given with MLV. Finally, there was a significant effect of the BHV-1 vaccines on the antileukotoxin titers. This interaction could have been due to synergism between the PhV and the gIV; however, in previous studies we have not seen any increase in antileukotoxin antibodies by vaccinating calves simultaneously with PhV and gIV (or with other subunit vaccines in the same adjuvant), compared to calves vaccinated with PhV only (unpublished observations).

The MLV vaccine used in this trial contained attenuated BHV-1 and PI-3. Either one or both of these viruses may have interfered with the response to the PhV. However, wild type BHV-1 can be immunosuppressive (44) and modified live BHV-1 vaccines may have the potential to interfere with other vaccines given simultaneously (45). Unfortunately, since we were not allowed to leave a control group of calves unvaccinated for BHV-1, we can only speculate that the interaction between BHV-1 vaccines and PhV was antagonistic between MLV and PhV, and not synergistic between gIV and PhV.

One important consideration in interpreting these data is that all manufacturers of modified live virus vaccines recommend that the vaccine should be given prior to the risk of disease and only to unstressed, healthy cattle (46). Although it is common practice to use these vaccines at the time of feedlot entry, this is directly contrary to manufacturers' recommendations. It has not been shown that there is an interaction between BHV-1 vaccines and other vaccines when they are used appropriately in a pre-immunization program. Generally, the accepted principles of immunology suggest that vaccines should be administered prior to the risk of disease in unstressed cattle.

This suspected antagonistic interaction of MLV against PhV may explain some of the inconsistency seen with other *P. haemolytica* vaccines in previous field trials. In one trial, in which protection was demonstrated with a culture supernatant *P. haemolytica* vaccine, each calf was vaccinated twice within the first four days of arrival at the feedlot (13). The first vaccination was given with MLV and the second was given without MLV. In a subsequent feedlot trial in which the same vaccine was not successful in reducing BRD, the vaccine was given in conjunction with a MLV (12). In one small trial in eastern Canada, which used a *P. haemolytica* vaccine with and without MLV, there appeared to be a trend of negative interaction between the two vaccines; however, the sample sizes were

relatively small and the differences were not significant (11).

Since no calves were left unvaccinated for BHV-1, and there were no obvious signs of clinical IBR, it was not possible to tell if there was limited exposure to BHV-1 or if both vaccines were effective in preventing IBR. Titers to gIV glycoprotein and gI glycoprotein at day 0 indicated that 12.5% of the calves had either passive antibodies or were exposed to vaccine or wild type BHV-1 prior to feedlot arrival. The gIV vaccine does not contain the BHV-1 gI glycoprotein and therefore the fact that 18.3% of the gIV group calves developed gI titers suggests exposure to either wild type virus or transmission of the attenuated BHV-1 strain from the MLV-vaccinated calves (Table 4).

One problem with PhV vaccination on arrival at the feedlot is that calves may already be sick from infection with *P. haemolytica*. We were interested in testing the PhV vaccine's efficacy in preventing *P. haemolytica* disease, not in treating existing disease. The exclusion of calves that were febrile at feedlot arrival and the prophylactic antibiotic treatment of all calves on the trial helped to reduce the number of calves that were already sick with *P. haemolytica* prior to the time when an immune response to the vaccine could take effect and be useful for the prevention of BRD (47,48). The use of prophylactic medication and temperature screening at arrival have been widely adopted in the health management strategies for fall placed auction market calves in Western Canada (49). Appropriate vaccination of calves prior to feedlot entry could eliminate the need for prophylactic antimicrobials.

The combination of PhV and gIV vaccines did not significantly reduce the level of overall mortality. Crude or overall mortality is a less specific efficacy outcome measure of a *P. haemolytica* vaccine trial than BRD morbidity, BRD mortality, or fibrinous pneumonia mortality. More than half of the crude mortality was due to disease that we would not expect to be prevented by a *P. haemolytica* vaccine. The least specific mortality outcome measure (crude mortality) had the lowest magnitude of efficacy when compared to more specific measures such as BRD mortality or fibrinous pneumonia mortality. The use of less specific disease outcomes can reduce the magnitude of effect (the VE) observed in field studies, and may result in missing an important effect. This phenomenom is called nondifferential misclassification bias (50) and can be a problem in feedlot studies that rely on data where selection criteria are not clearly defined for BRD morbidity or when postmortem examinations are not conducted to determine the cause of death. In this study, we felt BRD morbidity (as defined), BRD mortality, and fibrinous pneumonia mortality were reasonably specific outcomes for testing the PhV and BHV-1 vaccines.

We conclude that vaccination with PhV and gIV vaccines, on arrival at the feedlot, made significant reductions in fibrinous pneumonia mortality, BRD mortality, and BRD morbidity. The possible antagonistic interaction of MLV with PhV in this trial raises concern about the use of modified live virus vaccines at the same time as other vaccines in stressed cattle. The development of these and other new vaccines, and a better understanding of interactions between vaccines and management practices, should lead to more successful BRD vaccination protocols for feedlot cattle.

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